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MATING FREQUENCY IN BUSH-BROWN BUTTERFLIES (NYMPHALIDAE: SATYRINAE)

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ABSTRACT. Samples of adult female *Mycalesis perseus* (Fabr.), *M. terminus* (Fabr.) and *M. sirius* (Fabr.) (Nymphalidae: Satyrinae) collected over a two-year period during 1989–1990 at Cardwell, north-eastern Australia, were dissected to evaluate their mating frequency based on spermatophore counts. The three species were polyandrous with some individuals mating up to four times. Mating frequency varied seasonally, particularly in *M. perseus*, and in all species the mean number of spermatophores correlated significantly with age-class (extent of wing-wear): 'older' females were more likely to contain more spermatophores than 'younger' females. Despite age effects, significant differences between species were still detected in spermatophore counts, but only among older females. These findings indicate that the variables of relative age and season need to be taken into account when analyzing and comparing mating systems between species of Lepidoptera, particularly tropical butterflies.

Additional key words: insemination, mating behavior, spermatophore number.

Spermatophore counts in Lepidoptera are used widely to determine the number of times a female has mated and infer aspects of the mating system employed (e.g., Burns 1968, Pliske 1973, Wiklund 1977, Ehrlich & Ehrlich 1978, Drummond 1984, Wiklund & Forsberg 1991). Accordingly, female mating systems have been broadly characterized as either monandrous, where all females mate only once during their lifetime, or polyandrous, where most females mate several times during their lifetime. This classification has provided a useful framework in evolutionary studies investigating sexual selection and the adaptive significance of multiple insemination (Svärd & Wiklund 1989, Wiklund & Forsberg 1991, Wiklund et al. 1993 and references therein). In particular, polyandrous species are of considerable interest because it has been shown that male-derived nutrients from spermatophores at mating may be incorporated into both eggs and soma of the mated female (Boggs & Gilbert 1979, Boggs 1981, Boggs & Watt 1981), and females of some species appear to benefit from these nutrients (Rutowski et al. 1987, Watanabe 1988, Oberhauser 1989, Wiklund et al. 1993, but see Boggs 1990 for review).

Satyrine butterflies (Nymphalidae) generally are regarded as being essentially monandrous, or sometimes weakly polyandrous, as multiple insemination is comparatively rare (Ehrlich & Ehrlich 1978, Braby & New 1989, Wiklund & Forsberg 1991). However, Lederhouse et al. (1989) documented some of the pitfalls of using spermatophore data in

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analyzing mating systems. They identified three variables—spermatophore size, wing condition, and date of capture—which may greatly influence the number of times a female mates and therefore distort interpretation of the overall mating system. Moreover, Braby (1995a) recently demonstrated that mean spermatophore number can vary seasonally in tropical satyrines, particularly in species like *Mycalesis perseus* (Fabr.) which breed seasonally—spermatophore counts tend to be higher during the breeding season (wet season) and lower when adults are in reproductive diapause (dry season). These factors need to be considered first before drawing conclusions about female mating systems in general, and before attempting to evaluate the potential role of spermatophores as a nutritional resource in addition to providing sperm.

The aim of this work is to investigate and summarize aspects of mating frequency and behavior in three species of *Mycalesis* Hübner, the 'Bush-browns,' from the Australian tropics. In particular, the effect of wing-wear as an approximate estimate of female age on mating frequency is examined.

Materials and Methods

Samples of adult female *Mycalesis perseus, M. terminus* (Fabr.) and *M. sirius* (Fabr.) were collected from a variety of lowland habitats at Cardwell (18°16'S, 146°02'E), north-eastern Queensland, over a two year period during 1989–1990, primarily to study aspects of their reproductive strategies and breeding phenology. The butterflies were killed immediately after capture and transported to the laboratory at Townsville for dissection of the abdomen and examination of the reproductive tract. The sampling procedure, number and frequency of specimens, and laboratory analysis are described by Braby (1995).

The mating state and degree of wing-wear were recorded for each female of each species. The number of spermatophores was counted after dissection of the bursa copulatrix to determine the number of times a female had mated. The extent of wing-wear, as a measure of relative age, was classified subjectively in one of three categories according to the degree of wing tattering and scale loss: fresh = margins entire with none or very few scales missing; slightly worn = margins of one or two wings slightly chipped, 0–5% scales missing; worn = margins of two or more wings chipped, >5% scales missing.

Results

Estimates of female mating frequency, based on spermatophore counts, for the three *Mycalesis* species at Cardwell are presented in Table 1. Pooling these samples over the two year period allows broad comparisons between the species. Two generalisations can be made

TABLE 1. Mating frequency based on spermatophore counts for three species of *Mycalesis*. Percentages given in parentheses. Mating frequency is the total mean number of spermatophores per female.

Species	No. females with spermatophore count of:					Total examined	Mating frequency
	0	1	2	3	4		
M. perseus	37 (10.9)	220 (64.7)	70 (20.6)	9 (2.7)	4 (1.1)	340	1.18
M. terminus	17 (5.8)	202 (68.7)	71 (24.1)	4 (1.4)	0 (0.0)	294	1.21
M. sirius	18 (5.3)	195 (57.2)	109 (31.9)	18 (5.3)	1 (0.3)	341	1.38

from these data. First, although more than half of the total number of females examined for each species contained one spermatophore, the three *Mycalesis* species were not strictly monandrous and a high frequency of multiple insemination (polyandry) was evident for each. The frequency of polyandry varied from 24.4% in *M. perseus,* (n=83 individuals mated more than once), 25.5% in *M. terminus* (n=75), to 37.5% in *M. sirius* (n=128). Second, differences in overall mating frequency (i.e., the mean number of spermatophores per female) between the species were highly significant (x^2=33.98, df=88, $p<0.001$). This was mainly attributable to a higher incidence of multiple (second and third) matings in *M. sirius,* a relatively higher proportion of virgins and a lower incidence of multiple (second) matings in *M. perseus,* and a relatively low incidence of multiple (third) matings in *M. terminus.* Both *M. perseus* and *M. terminus* were more similar in mating frequency, although the differences were weakly significant (x^2=10.82, df=4, p=0.029).

How meaningful are these comparative differences in *Mycalesis*? When the frequency distributions of the number of spermatophores were examined in relation to age-class (wing-wear category) an age effect was evident (Table 2). For each species, spermatophore counts were significantly different between the wing-wear categories (*M. perseus*: x^2=91.13, df=8, $p<0.001$; *M. terminus*: x^2=53.41, df=6, $p<0.001$; *M. sirius*: x^2=51.63, df=8, $p<0.001$). That is, mating frequency was correlated with age-class, being lowest in 'younger' females (fresh category) and highest in the 'older' age-class (worn category). More importantly, the differences in spermatophore counts between species detected in Table 1 (age not controlled) did not persist among the 'younger' age-classes when age was controlled (fresh category: x^2=9.25, df=4, p=0.055; slightly worn category: x^2=7.53, df=6, p=0.275) (Table 2). However, among the 'older' age-classes, spermatophore counts between the species were significantly different (worn category: x^2=19.48, df=8, p=0.013). The differences in the older age-class were largely attributable to a higher incidence of multiple matings (three spermatophores) in *M. sirius* and an associated lower frequency of third matings in *M.*

TABLE 2. The number of females (n) with 0, 1, 2, 3 or 4 spermatophores and the mean number of spermatophores for each wing-wear category for three species of *Mycalesis*.

Wing-wear category	Number of spermatophores	*M. perseus*		*M. terminus*		*M. sirius*	
		n	(%)	n	(%)	n	(%)
Fresh	0	29	(33.3)	13	(24.1)	12	(19.4)
	1	52	(59.8)	37	(68.5)	38	(61.2)
	2	6	(6.9)	4	(7.4)	12	(19.4)
	3	0	(0.0)	0	(0.0)	0	(0.0)
	4	0	(0.0)	0	(0.0)	0	(0.0)
	total	87		54		62	
	mean	0.75		0.83		1.00	
Slightly worn	0	7	(9.0)	4	(5.8)	5	(5.8)
	1	61	(78.2)	50	(72.5)	58	(66.3)
	2	10	(12.8)	15	(21.7)	24	(26.7)
	3	0	(0.0)	0	(0.0)	1	(1.2)
	4	0	(0.0)	0	(0.0)	0	(0.0)
	total	78		69		88	
	mean	1.04		1.16		1.21	
Worn	0	1	(0.6)	0	(0.0)	1	(0.5)
	1	107	(61.1)	115	(67.3)	100	(51.8)
	2	55	(31.4)	52	(30.4)	74	(38.4)
	3	8	(4.6)	4	(2.3)	17	(8.8)
	4	4	(2.3)	0	(0.0)	1	(0.5)
	total	175		171		193	
	mean	1.47		1.35		1.57	

terminus and single matings in *M. sirius*; curiously, there was also a relatively high incidence of fourth matings in *M. perseus*. Hence, despite age affects, differences in mating frequency between the three species were real but only significant among 'older' females with *M. sirius* (47.7% of worn females mated more than once; mean number of spermatophores per female 1.57) and *M. perseus* (38.3% of worn females mated more than once; mean number of spermatophores per female 1.47) having a higher frequency of polyandry than *M. terminus* (32.7% of worn females mated more than once; mean number of spermatophores per female 1.35).

Observations on the daily and seasonal incidence of matings for *M. perseus* and *M. terminus* recorded in the field during 1989–1993, between Cardwell and Townsville, are summarized in Fig. 1. In *M. perseus*, matings were recorded mostly in the afternoon and most frequently during the wet season-early dry season with an apparent peak in March-May. Very few pairs in copula were recorded during the late dry season (September-December). By contrast, *M. terminus* matings were recorded most frequently at midday, though the sample size was rather

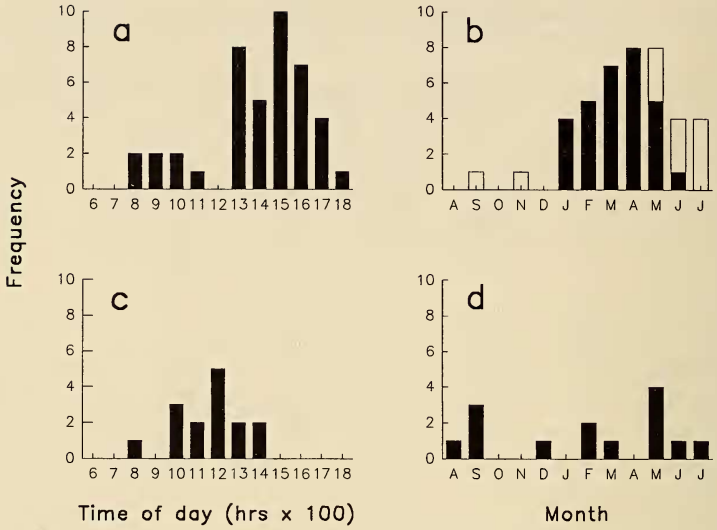

FIG. 1. Daily and seasonal variation in frequency of copulations in *M. perseus* (a, b) and *M. terminus* (c, d). Records are based on field observations accumulated for the period 1989–1993. Monthly records for *M. perseus* are divided into wet-season form (solid bar) and dry-season form (open bar).

small (n=15). The few matings recorded for *M. sirius* (n=11) precluded analysis of any daily or seasonal effects.

DISCUSSION

The relationship between mating frequency and age-class in Australian *Mycalesis* corroborates the findings of Lederhouse et al. (1989) for the tiger swallowtail *Papilio glaucus* Linnaeus in the United States. The three *Mycalesis* species are clearly polyandrous, but like *P. glaucus*, the frequency of mating increased significantly with wing-wear category, and a high proportion of virgins (>19% for each) was detected in the 'young' age-class (fresh category). Although the age of all females sampled was unknown, the assumption that wing-wear is broadly correlated with relative age is probably valid, although other factors such as inclement weather and predators may affect the quality of life, and hence wing-wear, to a certain degree (Lederhouse et al. 1989). However, it is noteworthy that significant (and similar) differences in mating frequency between *Mycalesis* were still evident once age affects were taken into account, although the differences persisted only among 'older' females.

The observations on diurnal and seasonal variation in mating observed in the field for *M. perseus* and *M. terminus* correspond well with their diurnal activity behavior and seasonal phenology (Braby 1995a, 1995b).

Both species have different peak activity periods during the day and they also show striking seasonal effects in mating state, particularly *M. perseus* in which spermatophore counts are highest during the breeding (wet) season. Hence, the effects of season and age are important variables in comparative studies. For example, it could be argued that *M. perseus* is strongly polyandrous, but only during its limited breeding period (Braby, 1995a), a finding which is not apparent when the count data are pooled for the two-year survey (Table 1 suggests *M. perseus* is only weakly polyandrous compared to *M. sirius*).

The effects of relative age (wing-wear) and season, together with the effects of spermatophore quality (size) (see Lederhouse et al. 1989) on mating frequency, underscore the need for caution when discussing the type of mating system favored by particular Lepidoptera (e.g., Ehrlich & Ehrlich 1978, Wiklund & Forsberg 1991). Only when these factors have been taken into account may it be reasonable to compare the mating strategies between species, or groups of species. If differences are found in insemination rate between species, or species groups, then adaptive explanations may be proposed.

Finally, why do older females tend to mate more frequently? Has the quantity of sperm from the first mating been insufficient to fertilize all eggs so that additional spermatophores are needed, or are females benefiting from male-derived nutrients, to enhance somatic maintenance and/or to increase reproductive effort? These questions may provide some fruitful areas of further investigation into the reproductive biology of Lepidoptera and the evolution of mating systems in general.

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LITERATURE CITED

BOGGS, C. L. 1981. Selection pressures affecting male nutrient investment at mating in Heliconiine butterflies. Evolution 35:931–940.

———. 1990. A general model of the role of male-donated nutrients in female insect's reproduction. Am. Nat. 136:598–617.

BOGGS, C. L. & L. E. GILBERT. 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206:83–84.

BOGGS, C. L. & W. B. WATT. 1981. Population structure of pierid butterflies. IV. Genetic and physiological investment in offspring by male *Colias*. Oecologia 50:320–324.

BRABY, M. F. 1995a. Reproductive seasonality in tropical satyrine butterflies: strategies for the dry season. Ecol. Entomol. 20:5–17.

———. 1995b. Seasonal changes in relative abundance and spatial distribution of lowland tropical satyrine butterflies. Austral. J. Zool. 43:209–229.

BRABY, M. F. & T. R. NEW. 1989. Adult reproductive biology of *Geitonera klugii* and *G. acantha* (Lepidoptera: Satyrinae) near Melbourne, Australia. Austral. J. Zool. 36:397–409.

BURNS, J. M. 1968. Mating frequency in natural populations of skippers and butterflies as determined by spermatophore counts. Proc. Natl. Acad. Sci. 61:852–859.

DRUMMOND, B. A. 1984. Multiple mating and sperm competition in the Lepidoptera, pp. 291–370. *In* Smith, R. L. (ed.), Sperm competition and the evolution of animal mating systems. Academic Press, New York.

EHRLICH, A. H. & P. R. EHRLICH. 1978. Reproductive strategies in the butterflies. I. Mating frequency, plugging and egg number. J. Kansas Entomol. Soc. 51:666–697.

LEDERHOUSE, R. C., M. P. AYRES & J. M. SCRIBER. 1989. Evaluation of spermatophore counts in studying mating systems of Lepidoptera. J. Lepid. Soc. 43:93–101.

OBERHAUSER, K. S. 1989. Effects of spermatophores on male and female monarch butterfly reproductive success. Behav. Ecol. Sociobiol. 25:237–246.

PLISKE, T. E. 1973. Factors determining mating frequencies in some New World butterflies and skippers. Ann. Entomol. Soc. Am. 66:164–169.

RUTOWSKI, R. L., G. W. GILCHRIST & B. TERKANIAN. 1987. Female butterflies mated with recently mated males show reduced reproductive output. Behav. Ecol. Sociobiol. 20:319–322.

SVÄRD, L. & C. WIKLUND. 1989. Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. Behav. Ecol. Sociobiol. 24:395–402.

WATANABE, M. 1988. Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus* L., in summer generations. J. Insect Behav. 1:17–30.

WIKLUND, C. 1977. Courtship behaviour in relation to female monogamy in *Leptidea sinapis* (Lepidoptera). Oikos 29:275–283.

WIKLUND, C. & J. FORSBERG. 1991. Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. Oikos 60:373–381.

WIKLUND, C., A. KAITALA, V. LINDFORS & J. ABENIUS. 1993. Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). Behav. Ecol. Sociobiol. 33:25–33.

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